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Toxicity of Pb-Contaminated Soil to Japanese Quail (*Coturnix japonica*) and the Use of the Blood–dietary Pb Slope in Risk Assessment

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ABSTRACT

This study relates tissue concentrations and toxic effects of Pb in Japanese quail (*Coturnix japonica*) to the dietary exposure of soil-borne Pb associated with mining and smelting. From 0% to 12% contaminated soil, by weight, was added to 5 experimental diets (0.12 to 382 mg Pb/kg, dry wt) and fed to the quail for 6 weeks. Benchmark doses associated with a 50% reduction in delta-aminolevulinic acid dehydratase activity were 0.62 mg Pb/kg in the blood, dry wt, and 27 mg Pb/kg in the diet. Benchmark doses associated with a 20% increase in the concentration of erythrocyte protoporphyrin were 2.7 mg Pb/kg in the blood and 152 mg Pb/kg in the diet. The quail showed no other signs of toxicity (histopathological lesions, alterations in plasma–testosterone concentration, and body and organ weights). The relation of the blood Pb concentration to the soil Pb concentration was linear, with a slope of 0.013 mg Pb/kg of blood (dry wt) divided by mg Pb/kg of diet. We suggest that this slope is potentially useful in ecological risk assessments on birds in the same way that the intake slope factor is an important parameter in risk assessments of children exposed to Pb. The slope may also be used in a tissue-residue approach as an additional line of evidence in ecological risk assessment, supplementary to an estimate of hazard based on dietary toxicity reference values. *Integr Environ Assess Manag* 2014;10:22–29. Published 2013 SETAC.#

Keywords: ALAD Protoporphyrin Liver Benchmark

INTRODUCTION

Concentrations of Pb in blood are used to estimate both exposure to Pb and its toxic effects in humans and in wildlife. When estimating hazards of Pb to children, assessors relate doses of Pb ingested to blood Pb concentrations which, in turn, provide a measure of the potential toxicity from Pb. The "intake slope factor" refers to the slope of the regression of blood Pb concentration on dose (ATSDR 2007). Knowing this slope could be useful also in assessing risk to wild birds at contaminated sites, where tissue concentrations may be combined with risk assessment models to derive cleanup levels. We suggest that the blood–diet Pb slope may be adapted to ecological risk assessments of wildlife and provide a second line of evidence of hazard based on tissue concentrations. In this study, we relate dietary Pb concentrations to tissue concentrations and to toxic effects in Japanese quail (*Coturnix japonica*) fed soil contaminated with Pb from mining and smelting. We also discuss the implications of the findings to ecological risk assessment.

Most of the literature on Pb poisoning in birds addresses the toxicity of manmade artifacts of Pb, such fishing sinkers, spent shot, and bullet fragments embedded in prey. At many Pb-contaminated sites, however, such as those near old mines and smelters, the Pb is dispersed in the soil. Soil ingestion is the main pathway of exposure to Pb in children (Mielke and Reagan 1998) and in domestic animals (Smith et al. 2009). Ingestion of soil and sediment is also key to understanding hazards to wildlife at contaminated sites. Although some Pb is present in dietary items at contaminated sites, the concentrations in soil tend to be so much greater that the hazards to wildlife depend mainly on the rate of soil ingestion and on the concentration of Pb in the soil. Ecological risk assessments of Pb to wild birds at contaminated sites have generally focused on the birds that ingest the most soil. American robins (*Turdus migratorius*) (Hansen et al. 2011) and the American woodcock (*Scolopax minor*) are highly exposed target species that have been used in estimating preliminary remedial goals for cleaning up soil.

Blood Pb concentrations have been used in monitoring Pb in wild birds, including swans (O'Connell et al. 2008) and other waterfowl (Henny et al. 2000), raptors (Henny et al. 1991, 1994; Rattner et al. 2008), gulls (Burger and Gochfeld 1997), and songbirds (Johnson et al. 2007; Hansen et al. 2011). Blood Pb concentrations are thought to be a measure of short-term exposure to Pb, but they are also used to estimate potential toxic effects (Buekers et al. 2009; Franson and Pain 2011). Blood Pb concentrations increase rapidly after dosing, within hours or days and, under constant exposure, remain approximately constant. In a 10-week study of mallards (*Anas platyrhynchos*) exposed to Pb, Heinz (1999) found that

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mean blood Pb concentrations at 5 weeks were within 5% of the mean concentrations at 10 weeks. Lead concentrations in avian livers, as in blood, depend mainly on current exposure, but Pb concentrations in kidneys increase with time (Anders et al. 1982).

Although we also measured Pb in liver and kidneys in our study, we were particularly interested in the quantitative relation between the exposure to Pb and the resulting blood Pb concentration. Previous studies on mammals had consistently found a nonlinear relation, the slope decreasing with concentration, whereas the limited published data available on birds suggest a linear relation for Pb. Knowing the relation is important not only for understanding the underlying toxicokinetics and for interpreting results from monitoring studies, but also for constructing quantitative ecological risk assessment models. Two bioindicators of Pb in the blood, the activity of red-blood cell delta-aminolevulinic acid dehydratase (ALAD) (Hoffman et al. 2000a) and the concentrations of erythrocyte protoporphyrin (Roscoe et al. 1979; Franson et al. 1986), were measured to quantify the toxicity associated with the blood Pb concentrations. A 50% decrease in the activity of ALAD of a population of birds or mammals is defined as injury in the Natural Resource Damage Assessment (1995) regulations (15 C. F. R. § 11.62 (f) (4) (v) (D)). An increase in erythrocyte protoporphyrin concentration is considered evidence of injury to the hematopoietic system (Anders et al. 1982).

Several controlled studies have examined the uptake of Pb from contaminated soil by mammals (Casteel et al. 2006), but less is known about uptake by birds. Pigeons (*Columbia livia*) dosed with capsules of soil contaminated with Pb from a small arms range (5400 μg Pb for 14 days) developed a mean blood Pb concentration of 0.89 μg/dL and an increase in erythrocyte protoporphyrin concentration (Bannon et al. 2011). Several feeding studies have been conducted on Pb-contaminated sediments from the Coeur d'Alene River Basin, which were dried, mixed into diets, and fed to waterfowl under controlled conditions (Heinz 1999; Hoffman et al. 2000a; Day et al. 2003). Connor et al. (1994) found that northern bobwhite (*Colinus virginianus*) fed a diet containing 8% of other sediment (4500 mg Pb/kg) from the Coeur d'Alene River Basin accumulated a mean of 1.26 mg Pb/kg (wet wt) in their blood.

Our objectives were to: 1) evaluate the toxicity of Pb-contaminated soil fed to Japanese quail, 2) estimate the benchmark doses (Filipsson et al. 2003) in diet and in blood associated with decreased activity of ALAD and increased concentrations of erythrocyte protoporphyrin, 3) estimate the blood–dietary Pb slope, and 4) suggest how this slope may be used in ecological risk assessment.

METHODS

Quail and diets

Surface soil for the study was collected with a spade from the Viburnum Trend, just southeast of the Buick Smelter, in the Mark Twain National Forest (37° 49′ 4.4″ N, 90° 44′ 16.7″ W), MO, on January 14, 2010. This soil was passed through a 2-mm sieve and a sample was sent to the Agricultural Analytical Services Laboratory of The Pennsylvania State University (University Park, PA) where soil pH, cation exchange capacity, organic matter content, and texture were determined. The Purina Game Bird Maintenance Chow (St. Louis, MO) selected for the study was a nutritionally complete mash diet for Japanese quail, with a minimum crude protein content of 12.5% and from 0.5% to 1% Ca. Because the quail might have preferentially selected larger particles of the mash and less soil from chow while feeding, we pelletized the diets, ensuring that the quail did ingest soil at the nominal rates. Water and cracked corn were added to the chow to produce a reference diet that could be pressed into stable pellets through a 6-mm die in a laboratory feed pelletizer. The pelletized control diet was 63% chow (dry wt), 27% corn (dry wt), and 10% water. Treated diets had the sieved soil added at the rates of 2%, 4%, 8%, or 12% as a fraction of the total dry wt of the diet. To form firm pellets, we added small amounts of water as required, as the soil content increased. Pellets were preserved frozen until they were fed to the quail. Samples of the soil and the mixed diets were sent to the Columbia Environmental Research Center in Missouri for analyses of metals.

Male quail were acquired from a breeding colony that had come from MA Ottinger, University of Maryland (College Park, MD). All procedures involving the handling of quail were reviewed and approved by the USGS Patuxent Wildlife Research Center's Animal Care and Use Committee. Twenty-three-day-old quail were acclimated to test conditions in separate stainless steel cages (23 cm wide × 38 cm deep × 33 cm height) for 5 days while weights were monitored. From those quail that maintained or increased their weight, we randomly assigned 30 4-week-old birds to the treatments. Each quail had access to its own set of semicircular feed and water cups hooked to the outside door panel, and feed and clean water were provided ad libitum. Automatic lights were set to provide a daily photoperiod of 16 h of light per day and temperature was maintained at 21° C. The trial ran for 6 weeks, beginning on November 22, 2010. Quail were observed and cared for daily.

At the end of the trial, quail were weighed and approximately 1.0 mL of blood was drawn from the jugular vein into a tuberculin syringe using a 25 gauge needle containing heparin (1000 USP units/mL; Sargent Pharmaceuticals, Schaumburg, IL) in the needle hub. Then the quail were euthanized with carbon dioxide followed by cervical dislocation. Subsamples of blood were apportioned as follows: 200 μL for chemical analysis, 75 μL for microhematocrit, 200 μL for ALAD, 50 μL for protoporphyrin, and 250 μL for testosterone. Cryotubes containing whole blood for ALAD assays were frozen in liquid N and then stored in an ultralow freezer at −80° C. Quail livers, kidneys, hearts, and testes were removed from the carcass and weighed. Portions of the blood, kidneys, and livers were sent to Columbia Environmental Research Center in Missouri for analyses of metals. Portions of the kidneys, livers, heart, and testes were preserved in 10% formalin and sent to the National Wildlife Health Center in Madison, Wisconsin, to be sectioned, stained, and examined for histopathological changes.

Biochemistry and pathology

Testosterone concentrations were determined in plasma samples by an enzyme-linked immunosorbent assay (ELISA) (DRG testosterone ELISA kit, EIA-1559; DRG International, Mountainside, NJ) with minor modifications of the manufacturer's protocol. Plasma had been isolated from the whole blood samples by centrifugation and then duplicate aliquots of 75 μL of plasma and enzyme-conjugated testosterone solution were mixed in wells of an antibody-coated microplate. The mixture was discarded after 90 min incubation at room temperature and the plate was washed. Substrate solution

was added to the wells, incubated for 20 min, and then the reaction was halted with the addition of stop solution. The optical density of each well was read at 450 ± 10 nm. The detection limit of the testosterone assay was 0.23 ng/mL, the recovery was 112%, the mean intra-CV was 10.9%, and the interassay CV was 16.3%. Proctodeal glands of male quail produce "foam," which is transferred to females during insemination. Foam production for each male was estimated qualitatively, on a scale of 0–3, based on the percent of fecal material with foam deposits; a score of 0 = no foam, 1 = 1%–5%, 2 = 6%–50%, and 3 = > 50% of fecal matter covered with foam deposits (Henry et al. 2012).

Red blood cell ALAD activity was measured as described by Burch and Siegel (1971) and as modified by Pain (1987), with enzyme activity optimized by use of pH 6.65 sodium phosphate buffer. One unit of activity equals an increase in absorbance of 0.100 at 555 nm wave length with a 1.0-cm light path/mL of red blood cells/h at 38° C. The average relative percent difference of the duplicate measures of activity was 6.6. Erythrocyte protoporphyrin concentration (μg/dL) was quantified with a hematofluorometer (AVIV Biomedical, Lakewood, NJ) modified according to Roscoe et al. (1979). The average relative percent difference of the duplicate concentrations was 3.7.

Tissue samples from liver, kidney, heart, and testes were fixed in 10% formalin, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin, as well as Ziehl-Neelson and Fite's acid-fast stains.

Metal analyses

All tissue samples were lyophilized to a constant weight and then homogenized to a powder consistency with a glass rod. A 40–60 mg dried subsample of blood, liver, or kidney was weighed into a 10-mL Teflon-lined, screw-cap borosilicate test tube and 1.0 mL subboiled HNO_3 was added. After a 1-h predigestion at room temperature, the tube was sealed and placed in a hot-block heater at 110° C for 30 min. The tube was cooled for 10 min, 0.2 mL high-purity H_2O_2 was added, and the tube was returned to the hot-block for 30 min. After cooling, the sample was diluted to a final volume of 10 mL for a final acid matrix of 10% HNO_3 (Brumbaugh et al. 2005). Subsamples of dried feed (0.25 g) were digested in sealed Teflon pressure vessels with 6 mL HNO_3 in a microwave digestion system. The samples were then diluted to 100 mL with deionized water for a final acid matrix of 6% HNO_3. Subsamples of dried soil (0.2 g) were digested in a sealed Teflon pressure vessel with 5.5 mL HNO_3 and 0.5 mL HCl in a microwave digestion system. The samples were diluted to 100 mL with deionized water for a final acid matrix of 5.5% HNO_3–0.5% HCl (Besser et al. 2007; Brumbaugh et al. 2007).

Concentrations of Cu, Zn, Cd, and Pb (blood, liver, kidney, and feed) and Al, Fe, Cu, Ni, Zn, Cd, and Pb (soil) were determined using a PE/SCIEX Elan 6000 ICP-DRC-MS (PerkinElmer Corporation, Norwalk, CT). Samples were automatically delivered to the ICP-MS by means of a software-controlled CETAC ASD-500 autosampler/autodiluter system (Cetac Technologies, Omaha, NE). All sample digestates were analyzed with 10× predilution by the autodiluter (Brumbaugh et al. 2005, 2007).

Method accuracy was determined through the use of standard reference materials (SRM): blood: Seronorm Trace Elements 201705 Whole Blood 3, Clinchek 8841 Whole Blood Control Level II; liver and kidney: National Institute of Standards and Technology (NIST) 1577 and 1577b bovine liver, National Research Council Canada (NRCC) TORT-1 lobster hepatopancreas; feed: NIST 1577 bovine liver, NRCC TORT-1 lobster hepatopancreas; soil: NRCC MESS-3 marine sediment, NRCC PACS-1 marine sediment, NIST 2709 San Joaquin soil. Three standard reference materials were run with each batch of tissue. Recoveries of Pb from SRMs ran with blood and kidney, and Cu, Zn, Cd, and Pb from SRMs ran with liver, ranged from 94% to 160% and averaged 102% ± 14% (standard deviation [SD]). Recoveries of Cu, Zn, Cd, and Pb from SRMs ran with feed samples ranged from 93% to 105% and averaged 99% ± 3%. Recoveries of Fe, Cu, Ni, Zn, Cd, and Pb from SRMs ran with soil ranged from 73% to 100% and averaged 93% ± 8%. The recovery of Al from SRMs ran with soil samples was considerably poorer (34% and 41%) because a total recoverable acid digestion procedure was used (similar to the US Environmental Protection Agency's (USEPA 1997) method 3051a), which yields acid insoluble residue and thus low recoveries for this element. Method accuracy was also accessed through the use of sample predigestion spikes or method spikes. Ranges and mean ± SD of spike recoveries for each matrix were as follows: blood: 96% to 102%, 100% ± 2.2%; kidney: 103% to 111%, 106% ± 4.3%; liver: 98% to 106%, 101% ± 2.1%; feed: 79% to 121%, 98% ± 6.4%; soil: 81% to 110%, 94% ± 7%. Method precision was evaluated by either duplicate or triplicate digestion and analysis of samples. Ranges and mean ± SD of relative percent differences (RPD) or percent relative standard deviations (%RSD) for each matrix were as follows: blood: 2.2% to 39%, 14% ± 14%; kidney: 6% to 12.5%, 9.7% ± 2.5%; liver: 0.4% to 7.3%, 3.6% ± 2.3%; feed: 1.6% to 32%, 10% ± 8.5%; soil: 0.6% to 34%, 8.5% ± 9.2%. Metal concentrations were reported as mg/kg, dry wt.

Statistics

Differences in mean metal tissue concentrations among treatment groups were compared with a Kruskal–Wallis analysis of variance on ranks. Protoporphyrin concentrations and ALAD activities were transformed to logs and then analyzed by an analysis of variance with the Holm–Sidak method to identify significant differences from control values. Blood, hepatic and renal Pb concentrations were linearly regressed on dietary Pb concentrations. All of these statistical calculations were run on SigmaPlot 9.0 and SigmaStat 3.1 software (Systat Software, San Jose, CA).

We used software (Benchmark Dose Software version 2.3.1) developed by the US Environmental Protection Agency (USEPA) (http://www.epa.gov/ncea/bmds/index.html) to estimate benchmark reference concentrations, expressed as Pb concentrations in both the diet and blood, associated with a predetermined benchmark response. The values of ALAD activity and erythrocyte protoporphyrin concentration were regressed on dietary Pb concentrations and on blood Pb concentrations using a series of regression equations. The best-fitting function for each variable was selected using the Akaike information criterion. Then the benchmarks associated with a 20% increase in the concentration of erythrocyte protoporphyrin and a 50% reduction in the activity of ALAD were estimated from the equations. The 20% decrease in protoporphyrin concentration was selected following the USEPA guidance (USEPA 2003) for developing soil screening levels and the 50% decrease in the activity of ALAD of a population of birds or mammals was selected because of its inclusion as an identified

Table 1. Mean ($N = 4$) Pb, Zn, Cd, and Cu concentrations in diets amended with increasing percentages of contaminated soil

Soil content of diet	Pb mg/kg (dry wt)	Cd mg/kg (dry w)	Zn mg/kg (dry wt)	Cu mg/kg (dry wt)
Control	0.12	0.07	74	11
2%	74	0.22	85	13
4%	125	0.31	76	11
8%	269	0.61	95	18
12%	382	0.84	95	18

injury in the Natural Resource Damage Assessment Regulations (15 C. F. R. § 11.62 (f) (4) (v) (D)).

RESULTS

Toxic effects

The experimental soil from Viburnum Trend, a loam, had a pH of 5.0, a cation exchange capacity of 14 (meq/100 g), and an organic matter content of 12%. It contained 3720 mg Pb/kg, the major contaminant at the site, 264 mg Zn/kg, 7.2 mg Cd/kg, and 98 mg Cu/kg, dry wt. The treated diets contained concentrations of Pb (74–382 mg/kg) that were consistent with but slightly below concentrations expected from their soil contents (Table 1). The adjusted *r*-square of the correlation between percent soil in the diet and the Pb concentration of the diet was 99%. The other metals measured in the diet were not likely to be toxic to the quail. The highest dietary concentrations of Zn and Cu in the study, in the 12% soil group, were less than twice the concentrations of those metals in the control diets (Table 1). Dietary concentrations of Cd showed a greater increase with soil content but, nonetheless, the 12%-soil diet contained only 0.84 mg Cd/kg. Hepatic Cd concentrations increased significantly with dietary soil content but hepatic Zn and Cu concentrations did not (Table 2). The highest observed mean hepatic Cd concentration, of 2.1 mg/kg, dry wt (Table 2), was well below the injury threshold (range of 45–70 mg Cd/kg, wet wt) suggested by Wayland and Scheuhammer (2011).

Quail gained an average of 48% of their weight (from 71 g to 105 g) over the 6 weeks and all birds appeared normal throughout the trial. Similar aged Japanese quail from the parent colony ate approximately 16 g of diet per day. The weight gains, ratios of organ weights (liver, kidney, heart, testes) to body weights, hematocrits, and foam production of the Pb-exposed quail were statistically indistinguishable ($p > 0.05$) from control values. Average plasma testosterone concentrations for all treatment groups were within 1.4–2.02 ng/mL, with no significant differences between the treated groups and the control ($F[4, 23] = 0.22$, $p = 0.93$). Testosterone concentrations were consistent with those measured in control birds from the colony; young, unpaired 8-week-old control males from the colony had a lower average plasma testosterone concentration of 0.92 ng/mL and actively breeding adult control males had values between 2.3–3.5 ng/mL. No histologic lesions were observed in testes and hearts. Lesions observed in livers (hepatocellular hydropic degeneration and lipidosis) and kidneys (proximal tubular epithelial cell vacuolation, necrosis, and hemosiderosis) bore no relation to treatment levels and were probably not caused by exposure to Pb. We observed neither the nephrosis reported in Pb-poisoned Japanese quail (Almansour 2008) nor the renal nuclear inclusion bodies reported in Pb-poisoned northern bobwhite (Beyer et al. 1988).

Lead had physiological effects on the blood; all quail groups exposed to Pb-contaminated soil had a significantly lower mean ALAD activity than did the control group ($p < 0.05$). Activity of ALAD decreased with dose, from 222 units in control quail to 5 units in the 12% soil group (Table 3). The benchmark doses associated with a 50% reduction in ALAD activity were 0.62 mg Pb/kg in the blood and 27 mg Pb/kg in the diet, based on the exponential function. Mean protoporphyrin concentrations increased at higher Pb doses, to approximately double the control mean at the highest dietary Pb concentration. The benchmark doses associated with a 20% increase in the concentration of erythrocyte protoporphyrin were 2.7 mg Pb/kg in the blood and 152 mg Pb/kg in the diet, based on the Hill function. The lower 95% confidence limits of the benchmarks were slightly lower: ALAD, 0.58 mg Pb/kg in the blood and

Table 2. Hepatic metal concentrations in Japanese quail fed Pb-contaminated soil at 0% to 12% in the diet for 6 weeks

Soil content of diet	Hepatic Pb mg/kg (dry wt)	Hepatic Cd mg/kg (dry wt)	Hepatic Zn mg/kg (dry wt)	Hepatic Cu mg/kg (dry wt)
Control	0.01	0.25	66	15
2%	3.3	0.61	62	14
4%	7.3	1.0	67	15
8%	9.1	1.3	62	14
12%	12	2.1	68	16
Significant by Kruskal–Wallis test at $p < 0.05$, $N = 6$ per group	Yes	Yes	No	No

Table 3. Tissue concentrations of Pb (mg/kg, dry wt), erythrocyte ALAD activity, and protoporphyrin concentrations in Japanese quail fed Pb-contaminated soil at 0%–12% in the diet ($N = 6$ per group)

Treatment	Blood Pb	Hepatic Pb	Renal Pb	ALAD activity [a] (units [b])	Protoporphyrin [a] (μg/dL)
Control	0.12	0.01	0.03	222	342
2% soil	1.4	3.3	11	37*[b]	339
4% soil	2.5	7.3	18	13*	374
8% soil	3.2	9.1	33	7*	626*
12% soil	5.4	12	48	5*	687*
Statistically significant ANOVA ($p < 0.05$)	Yes [c]	Yes [c]	Yes [c]	Yes	Yes

[a]One unit of activity equals an increase in absorbance of 0.100 at 555 nm wave length with a 1.0-cm light path/ml of red blood cells/h at 38° C.
[b]Asterisk signifies a significant difference from the reference value by the Holm–Sidak method at $p < 0.05$, performed after a significant ANOVA. Units of ALAD activity were log-transformed.
[c]Kruskal–Wallis analysis of variance on ranks.

25 mg Pb/kg in the diet; protoporphyrin, 2.5 mg Pb/kg in the blood and 117 mg Pb/kg in the diet.

Relation of tissue Pb concentrations to dietary Pb concentrations

Concentrations of Pb in blood, liver, and kidney increased with dietary soil content (Table 3 and Figure 1), with adjusted *r*-squares of 0.97 (blood), 0.96 (liver), and 0.99 (kidneys). All of the relations appeared to be linear. The slope of the regression (Figure 1) of blood Pb concentration divided by dietary Pb concentration was 0.013 (mg Pb/kg of blood, dry wt, divided by mg Pb/kg of diet). Blood–dietary Pb slopes were calculated from data published in 8 other Pb dietary feeding studies on wildlife for comparison (Table 4). All of these slopes apply to chronic exposures to Pb in nutritionally complete commercial diets and were based on at least 3 pairs of data. The slope for our Japanese quail was converted to wet wt (0.0027 mg Pb/kg blood, wet wt, divided by mg Pb/kg of diet), based on a measured 79% moisture content of the blood. The median of the slopes was 0.0036 mg Pb/kg blood, wet wt, divided by mg Pb/kg diet and all of the values were between 0.0015 and 0.0072.

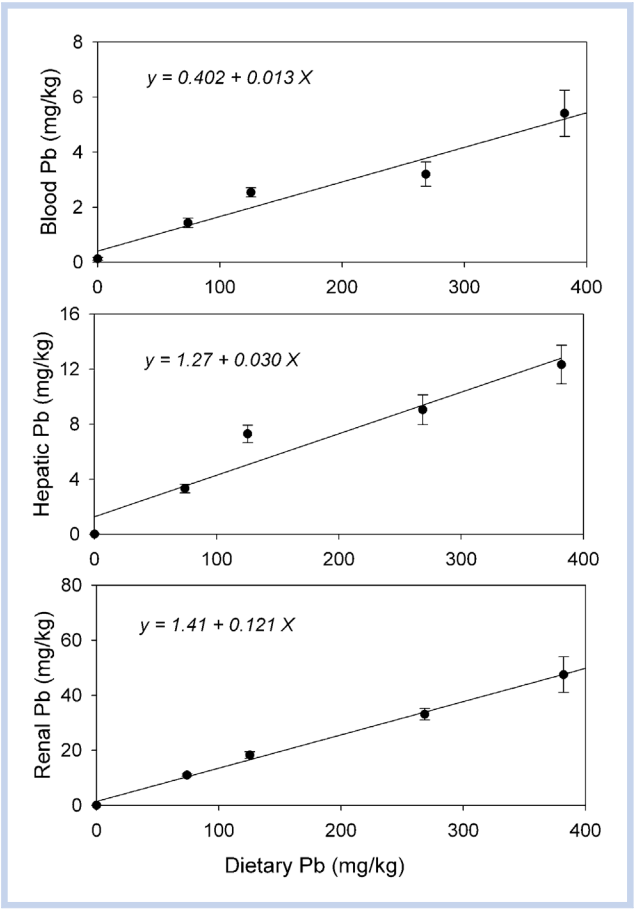

Figure 1. Regressions of blood, hepatic, and renal Pb concentrations on dietary Pb of Japanese quail fed increasing percentages of contaminated soil. Note that the regressions are linear and that the control concentrations are close to zero. The adjusted *r*-squares are 0.97 (blood), 0.96 (liver), and 0.99 (kidneys), all significant at $p < 0.01$.

DISCUSSION

Toxic effects

At low levels of exposure to Pb, Japanese quail, like mallards, experience reduced activity of ALAD and, when exposed to greater Pb concentrations, an increase in erythrocyte protoporphyrin (Heinz 1999). The lack of other observed signs of toxicity in the quail is consistent with earlier studies suggesting that galliforms (turkeys, pheasants, quail, and chickens) are relatively resistant to Pb poisoning (Franson and Pain 2011), except for egg production. In a 5-week feeding trial, for example, Japanese quail developed anemia and weighed less than controls, but only when fed 500 mg Pb/kg as Pb acetate (Morgan et al. 1975). Weights of their testes were reduced only at a dietary Pb concentration of 1000 mg/kg. Lead nitrate and Pb subacetate caused no overt signs of toxicity in 2-week-old Japanese quail fed a diet containing 5000 mg Pb/kg over 5 days (Hill and Camardese 1986). In addition, tissue Pb concentrations associated with injury tend to be higher in northern bobwhite quail than in other avian species; median Pb concentrations associated with death reported in livers (102 mg/kg) and kidneys (185 mg/kg) of bobwhite were greater than corresponding concentrations in livers (22–44 mg/kg) and kidneys (22–140 mg/kg) of 5 other avian species (Beyer et al. 1988). The rate of egg production in Japanese quail, however, is extremely sensitive to Pb; as little as 1 mg Pb/kg in the diet significantly decreases the number of eggs laid (Edens et al. 1976). In contrast, as much as 1000 mg Pb/kg in the same

Table 4. Blood–diet slopes of regressions (mg Pb/kg blood, wet wt – mg Pb/kg diet) of Japanese quail fed Pb in their diets

Species	Pb Source	Dietary Pb mg/kg	Blood Pb mg/kg (wet)	Slope	Reference
Japanese quail	Mining and smelting soil (MO)	0.12, 74, 125, 269, 382	0.026, 0.30, 0.53, 0.67, 1.14	0.0027	This study
Canada Goose (goslings) [a]	Coeur d'Alene sediment	3, 414, 828, 1656	0.03, 0.68 1.61, 2.52	0.0015	(Hoffman et al. 2000b)
Mallard duckling	Coeur d'Alene sediment	3, 417, 831	0.03, 1.41, 2.56	0.0031	(Hoffman et al. 2000a)
Mallard (subadult)	Coeur d'Alene sediment (exp. 2)	8.7, 642, 1284	0.09, 3, 6.8	0.0053	(Heinz 1999)
Mallard (subadult)	Coeur d'Alene sediment (exp. 1)	1.5, 103, 207, 414, 828	0.04, 0.99, 1.7, 2.9, 6.1	0.0072	(Heinz 1999)
Mallard	Pb nitrate	1,5,25	27, 55, 138	0.0049	(Finley et al. 1976)
Mute swan [b]	Coeur d'Alene sediment	5.8, 460, 850	0.20, 1.28, 2.3	0.0025	(Day et al. 2003)
Broiler chickens	Pb acetate	3.9, 4.9, 14, 104	0.04, 0.13, 0.18, 0.50	0.0040	(Bakalli et al. 1995)
Deer mice [c]	Pb acetate	0.12, 1.3 13, 133	0.006, 0.013, 0.43, 0.50	0.0036	(McBride 2007)

[a]*Branta canadensis*.
[b]*Cygnus olor*.
[c]*Peromyscus maniculatus*.

study did not reduce growth in males. The USEPA (2005) calculated an ecological soil screening level (eco-SSL) of 11 mg Pb/kg of soil based on reproductive effects observed in Japanese quail and chickens fed extremely low concentrations of Pb (Edens and Garlich 1983). This concentration of 11 mg/kg is below the national average of 19 mg Pb/kg in surficial materials (Shacklette and Boerngen 1984) and implies that background concentrations of soil Pb are toxic to wild birds that ingest a large amount of soil as they feed. In general, the rate of egg production is not a sensitive endpoint to Pb toxicity in birds other than galliforms; treatment of mallards with a number 4 Pb shot did not affect egg production (Finley and Dieter 1978); a dose of 1 number 8 Pb shot caused some mortality in mourning doves (*Zenaida macroura*) and reduced fertility but did not reduce productivity (Buerger et al. 1986); studies on kestrels (*Falco sparverius*) fed up to 50 mg Pb/kg (Pattee 1984) and on ringed turtle doves (*Streptopelia risoria*) fed up to 10 μg Pb/mL of water (Kendall and Scanlon 1981) also detected no reduction in productivity. We conclude that although Japanese quail provide a useful model for studying the kinetics of Pb in birds and hematological endpoints (ALAD, protoporphyrin), they seem to be a poor surrogate species for wild birds in general in ecological risk assessments of Pb.

Relation of blood Pb concentrations to dietary Pb concentrations in birds

The regression of the concentration of a contaminant in blood to an external dose provides a means to relate environmental exposure to the internal, or absorbed, dose. Knowing the absorbed dose is useful for evaluating both exposure and potential toxic effects.

The relation between blood Pb concentration and dietary Pb concentration has been more thoroughly studied in mammals than in birds. Researchers studying mammals have found greater relative absorption of Pb from the diet at low concentrations, leading to a concave (decreasing slope) curvilinear relation (ATSDR 2007). This greater absorption may be caused by active absorption of Pb associated with Ca transport across the gut. At higher concentrations of Pb, saturation of red blood cells with Pb may occur (ATSDR 2007). In contrast, however, the regressions of Pb in blood, liver and kidneys for Japanese quail are all linear. Previous studies on waterfowl fed Pb-contaminated river sediments from the Coeur d'Alene River Basin also suggest a linear relation for birds, even at blood Pb concentrations as high as 6 mg Pb/kg, wet wt (Beyer et al. 2000). It is possible that increased absorption of Pb could occur at a very low exposure but, for the purposes of risk assessment, it is reasonable to assume linearity, which simplifies modeling of Pb in birds.

In studies meant to protect humans from Pb, risk assessors often rely on the "intake slope factor," which is the slope of the regression of blood Pb concentration to dose. Sometimes this parameter is replaced by the product of the estimated fraction of Pb absorbed (bioavailability) and the "biokinetic slope factor," which depends on the kinetics of Pb once it is absorbed and interacts with blood (ATSDR 2007). Intake slopes for human health are expressed as the weight of Pb consumed, rather than on the concentration in the diet, as in our study, which prevents the direct comparison between the intake slope and our blood–dietary slope. Because assessment models for humans are designed to protect the most susceptible children in a population, the laboratory studies supporting the assessments generally exposed animals to Pb-contaminated soil on an empty stomach, which increased the rate of absorption. Humans were found to absorb 8.2% of a dose of Pb taken with food and 35% of a dose of Pb taken without food (Rabinowitz et al. 1980). In contrast, we assumed that wild birds would ingest soil mainly while feeding and incorporated contaminated soil into the diet.

The blood–dietary Pb slope for our Japanese quail of 0.0027 (mg Pb/kg blood [wet wt] ÷ mg Pb/kg diet) was similar to most

of the other slopes shown in Table 4, which were generally between 0.0025 and 0.0053.

The variation is well below that associated with toxicity reference values of Pb (USEPA 2005). The consistency among the values was unexpected, given the range of species and the variety of experimental exposures in the studies. We do not know how much of the total variation in these values is associated with each of the variables expected to be relevant—the species, diet, and bioavailability of the Pb. The blood–dietary Pb slope should vary directly with the bioavailability of the Pb, which depends on the chemical form of the Pb, as well as on the chemical properties of food and soil that inhibit absorption from the gut. Hoffman et al. (2000a) reported that Pb from contaminated sediment in feed was 44% as available as Pb acetate in feed when fed to ducklings. Blood Pb concentrations were found to be greater when waterfowl were fed suboptimal diets (Hoffman et al. 2000a; Day et al. 2003), possibly due to nutritional deficiencies, such as a reduced level of Ca (Scheuhammer 1996), or to a greater rate of ingestion. Compounds may bind Pb to soil and sediment; the addition of phosphoric acids to Pb-contaminated sediments decreased the rate of absorption by subadult mallards (Heinz et al. 2004; Furman et al. 2006). If the relative bioavailability of Pb in a soil were known, then the blood–dietary Pb slope could be adjusted for use in risk assessment.

Ecological risk assessments generally relate a toxicity reference value to an estimated exposure. However, if an exposure to Pb had been estimated, then the blood Pb concentration could be estimated from the blood–dietary Pb slope and the toxicity could be estimated with a tissue residue approach; Buekers et al. (2009) have gathered together critical blood Pb values for many avian species and Franson and Pain (2011) have reviewed the topic of interpreting Pb concentrations in avian tissues. Supplementing a risk assessment based on toxicity reference values with a tissue-residue approach as a second line of evidence should reduce the uncertainty associated with a risk assessment. Estimates of exposure at contaminated sites are generally made from biomagnification factors in the literature or rarely by analyzing dietary items, stomach contents or fecal samples. If the blood–dietary slope for a bird were known, then analyzing avian blood from a contaminated site would provide a means to estimate exposure as well as to ground-truth an assessment. When collecting and analyzing blood samples from the field, it is important to include those species that are expected to ingest the most soil. The length of time the birds have been present and the soil Pb concentrations throughout the feeding range are relevant. Although the blood Pb concentrations from the field should integrate exposure across a heterogeneous site, the variability in blood Pb concentrations in birds from the field would be expected to be much greater than that observed under controlled conditions and so it is important to have an adequate sample size. Significant species-specific differences in blood–dietary slopes would introduce errors into the estimates of exposure.

Because the blood–dietary Pb slope is based on soil ingestion, we may also predict the soil ingestion rate, using Pb as a tracer for soil if we know the mean blood Pb concentration and the soil Pb concentration at a site. This would provide us with a means to estimate a bird's exposure to other metals, such as U, Ni, Fe, or Al, whose main pathway of exposure is also mainly through soil ingestion; the exposure to Al, for example, would be simply the ratio of the soil concentrations of Al to Pb multiplied by the exposure to Pb. This simple method could be especially useful at the screening level in risk assessments when several contaminants are considered.

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